**Lab five:Microstimulation**

**Overview**

**In this lab you will:**

1. learn how to prepare a cockroach leg for microstimulation;
2. testhow different stimulations cause spikes in motor neurons;
3. observe the difference between sensory and motor spiking patterns; and
4. investigate how different types of stimulations affect the generation of action potentials in a cockroach leg preparation.

**Objectives**

**Before doing this lab you should understand:**

* how neurons communicate with muscle cells
* how electrical stimulation can cause a muscle contraction

**After doing this lab you should be able to:**

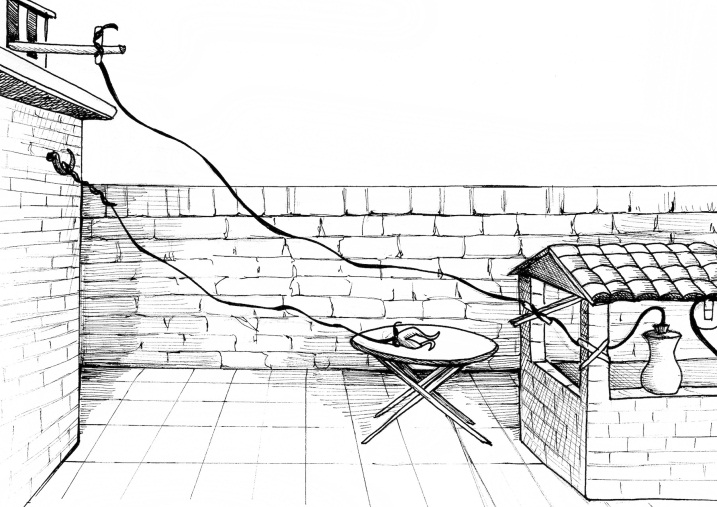
* explain how a nervous system controls muscle movement
* describe how distinctmovements are caused by specific spiking patterns
* design an experiment to map ideal frequencies for stimulation

**EQUIPMENT**

* SpikerBox with audio cable adapter
* computer with Audacity installed
* cockroach
* ice water or access to a freezer
* dissection scissors
* toothpick
* microstimulation electrodes
* electrical signal generator (iPhone or MP3 Player)
* Y-splitter (optional)
* external speaker (optional)

**Introduction**

Long before scientists were able to record spikes, they were able to stimulate the nervous system using batteries (Leyden Jars). Since nerves use electricity to communicate, they can be manipulated with electricity as well. Luigi Galvani, an Italian scientist in the 1700’s, discovered that electricity applied to the nerves of frog legs caused the large muscles to twitch.

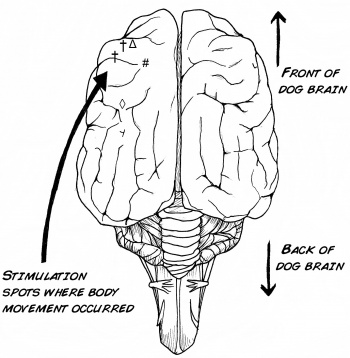
Such discoveries led to debates at the time as to whether “animal electricity” was different from the electricity during lightning storms. Galvani also tested this by hanging frog legs off his back porch during thunderstorms & watching the legs twitch.These results were also inspiration for Mary Shelley’s “Frankenstein:”

“Perhaps a corpse would be re-animated; galvanism had given token of such things: perhaps the component parts of a creature might be manufactured, brought together, and endured with vital warmth.”

-Mary Shelley, Introduction to Frankenstein

Eventually the scientific community agreed and discovered that while electricity can indeed stimulate nervous system and muscle tissue, the tissue itself generates electricity. This led to the beginnings of contemporary neuroscience, which you are studying today.

We learned about action potentials (APs) and neuron-neuron synapses in Lab One, but the galvanic muscle responses are a result of synapses between motor neurons (MNs) and a skeletal muscle, or neuromuscular junctions. Unlike the convergent connections on central nerve cells, a postsynaptic muscle cell is normally innervated by just a single presynaptic MN at a specialized region of the muscle membrane called the end-plate. Acetylcholine (ACh) is released by the axon terminal from the MN, which directly opens voltage-gated Ca+2 ion channels in the muscle membrane that allows Ca+2 to enter the terminal with each action potential. Motor neurons excite the muscle by opening ion channels at the end-plate, producing a large amplitude end-plate potential that rapidly activates voltage-gated Na+ channels and produces an action potential that propagates along the muscle fiber and generates movement.



In another famous experiment, German medical scientists Eduard Hitzig and Gustav Fritsch in 1870 applied electric current to the exposed cerebral cortex (wrinkly part of brain) in dogs in their kitchens (yes, it was odd even back then) showing that stimulation of different parts of the brain can cause different types of movements.

Similarly, it has been shown that stimulation of the nerve cord can produce rhythmic patterned outputs to leg muscles, termed central pattern generators (CPGs). T. Graham Brown demonstrated as early as 1911 that these coordinated spiking patterns can induce the basic muscle responses of stepping without the need of descending commands from the cortex.



Today, such central-stimulation/motor-sensory-output techniques are used in patients, most notably those afflicted with Parkinson’s disease.By inserting a small, long electrode into a specific part of the brain called the subthalamic nucleus, the shaking and tremors associated with the disease can be lessened. Sometimes there are side-effects though, like increased gambling & other compulsive behaviors.

Today, some advanced research groups are designing small chips that stimulate the nerves of the eye as a cure for blindness.

**SETUP**

**Microstimulation Electrode**

The microstimulation electrode will act as a conduit between your electrical signal generator and the cockroach. The electrode will consist of an audio cable that plugs into your signal generator as a means to deliver the stimulation to the cockroach. You can choose to solder either pins to your audio cable, similar to those used in Lab 1, or use alligator clips that can be attached to the electrodes that come with your SpikerBox.

**Electrical Signal Generator (ESG)**

There are many programs that can generate electrical stimuli ideal for this lab. If you are using an iPhone or iPad, these free apps can be found at the iTunes store at the following links**:**

[**AudioSigGen**](http://itunes.apple.com/us/app/audiosiggen/id347248794?mt=8)

[**FreqGen**](http://itunes.apple.com/us/app/freqgen/id325832805?mt=8)

If you are using a PC, you can use this online software:

[**Rhintech**](http://www.rhintek.com/tutorial/Frequency/index.php)

Additionally, you can simply download various frequencies as MP3s and play them through any MP3 player. Here is a website from which you can download free frequencies appropriate for this exercise:

[**TestSounds**](http://www.testsounds.com/)

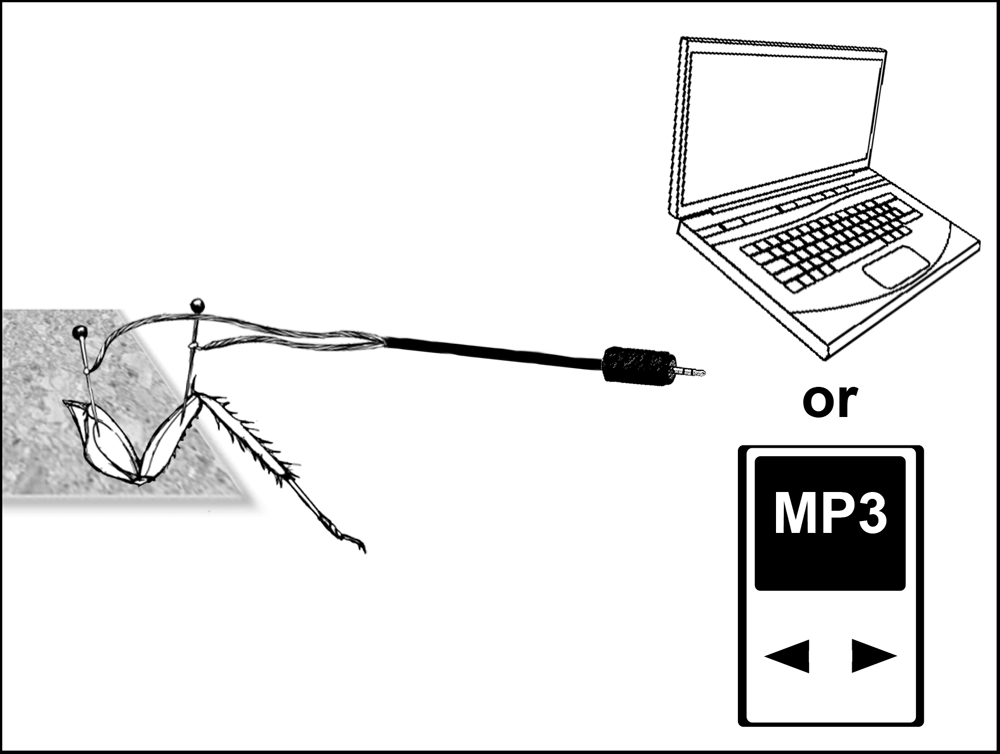
**PROCEDURE**

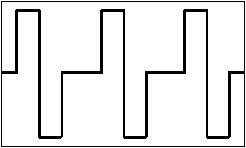
**Exercise 1: Cockroach Leg Microstimulation Preparation**

1. Prepare a cockroach leg as described in Lab 1.

2. Attach the microstimulation electrode. If using the alligator clip electrode, attach the clips to the SpikerBox recording electrodes inserted into the coxa and femur. If using a direct electrode, place the electrodes into the coxa and femur of your cockroach leg.

3. Plug the Microstimulation Electrode into an ESG such as a computer or MP3 player.





4. If you are able to, program your ESG to produce square waves.

5. Set your ESG to 50% volume (amplitude) and generate a signal with a frequency of 200 Hz (200 repetitions per second).

6. Play the tone and observe the cockroach leg. If the leg moves in response to the stimulation, continue on to Exercise 2. If the leg does not move, continue to Step 7.

7. If the leg does not move in response to the stimulus, ensure you have your electrode plugged into the audio out jack and that the tone is being produced. You should be able to hear a 200 Hz tone at half volume with **EXTERNAL SPEAKERS.** If you do not hear a tone, you may need to adjust your audio settings.

8. If you can produce a tone with your ESG, then ensure that your cockroach leg is alive and you have your electrodes in the proper place. Using the protocol in Lab 1: Exercise 1, use your SpikerBox electrodes and speaker to ensure spikes are being produced by the leg. Ensure you have placed your electrodes in the coxa and femur.

9. Ensure the soldering on your electrodes is intact and that your electrodes are not touching each other. Consult with your teacher if you continue to have problems stimulating your cockroach leg.

**Exercise 2: Response of Cockroach MNs to Amplitude and Frequency**

Now that you have a functioning Microstimulation Apparatus, it is time to measure the frequency and amplitude response properties for the cockroach motor neurons (MNs). This will be achieved by simple observation of your leg while stimulating it with an array of different tones.

1. Begin stimulation of the leg at the lowest frequency (20 Hz) and volume (lowest amplitude your device is capable of) shown in Table 1. Keep in mind, if you were to try to listen to the output of your ESG, you may not be able to hear a tone of 20 Hz no matter how loud you make it. Human hearing ranges between 20 Hz and 20,000 Hz (20 kHz), although there is variation between individuals.

**CAUTION: DO NOT USE HEADPHONES FOR THIS EXPERIMENT IF THE VOLUME OF YOUR *ESG* IS ABOVE 50% AS YOU MAY CAUSE DAMAGE TOYOUR HEARING. ALWAYS USE AN EXTERNAL SPEAKER.**

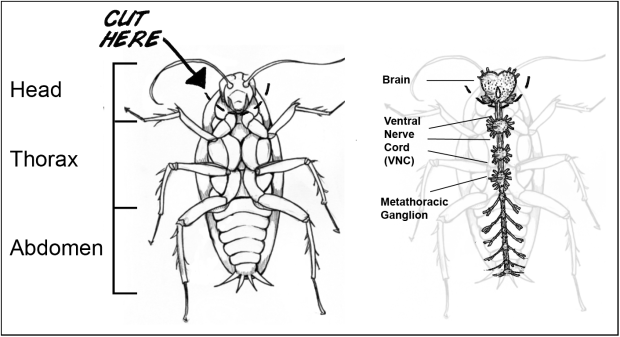
2. Using Table 1 as a guide, test the responsiveness of your leg to all amplitudes of each frequency. Mark each trial with either a (+) or (-) to signify whether the leg moved in response to the stimulation. If your ESG does not produce the frequencies listed in Table 1, use whatever frequencies you have and adjust Table 1 accordingly.

**Table 1. Response by Frequency and Amplitude**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Frequency** | **Min Output** | **¼ Output** | **½ Output** | **¾ Output** | **Max Output** |
| **20 Hz** |  |  |  |  |  |
| **40 Hz** |  |  |  |  |  |
| **80 Hz** |  |  |  |  |  |
| **150 Hz** |  |  |  |  |  |
| **200 Hz** |  |  |  |  |  |
| **1000 Hz** |  |  |  |  |  |
| **2000 Hz** |  |  |  |  |  |
| **3000 Hz** |  |  |  |  |  |
| **5000 Hz** |  |  |  |  |  |
| **10,000 Hz** |  |  |  |  |  |

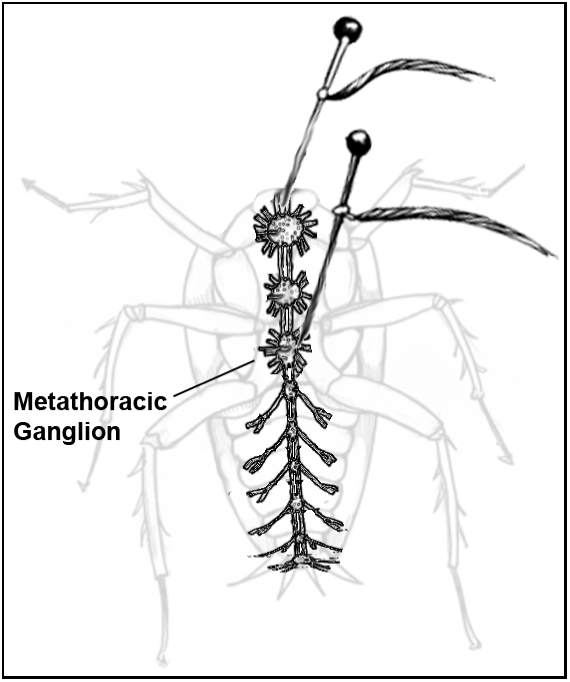
**Exercise 3A: Recording and Spike Patterns from Cockroach Ganglia**

**Note: This experiment requires a cockroach to be sacrificed**

1. Place a cockroach in ice water or a freezer.When the cockroach has been anaesthetized, place it on your lab bench.

2. Using your dissection scissors, decapitate the cockroach at the junction between the head and the thorax. Place some petroleum jelly on the cut.

3. Insert your SpikerBox recording electrode through the thorax of the cockroach into the **metathoracic ganglion**. Try to place the electrode slightly to one side of the ganglion. Stick the ground electrode into the thorax where the head used to be.



4. Plug your SpikerBox into your computer, turn it on, and start Audacity. **Begin recording** from the metathoracic ganglion.**Adjust the Y-axis** of the recording so that the spikes observed are maximized. **Adjust the X-axis** (using the magnifying glass tool in the menu bar) so that you can differentiate bursts directly related to movements.

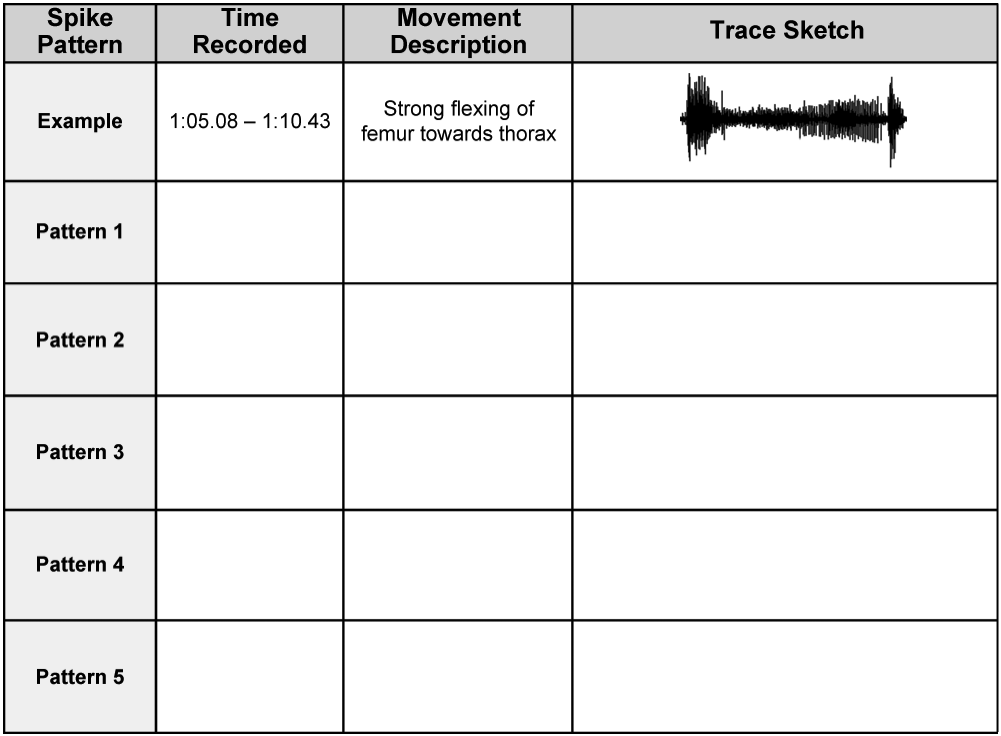
5. For several minutes, observe recordings from the cockroach and take note of how they correspond to movements of the metathoracic legs. As the connections between the ventral nerve cord (VNC) and the metathoracic legs are intact, action potentials are still able to propagate down motor neurons to the muscles. You should also be able to observe spontaneous movements from the cockroach. Keep in mind that you are likely to be observing three types of information while recording from the ganglion: a) motor information being sent from the ganglion to the leg muscles; b) sensory information being sent in to the VNC from the legs; and c) information being integrated and processed between ganglia along the VNC. Your goal is to identify spiking patterns that lead to motor movements.

6. Once you feel like you can see a relationship between specific leg movements and specific patterns of spikes, mark down the following pieces of data in the Table 2 for five different events: a) the time of the spikes; b) a description of the movement.

7. Stop recording from Audacity and find the spiking patterns that you noted in Table 2 in the trace. Highlight with your mouse and copy (Control-C) the small section of one spike series. Open a new Audacity file (Control-N) and paste the copies spike segment (Control-V). Do this for each of the 5 spike patterns from Table 2 so that you now have 5 new audacity windows open.

8. For each spike pattern, amplify the trace under **Effect-Amplify** in the menu bar. Briefly sketch each pattern in Table 2. Save each spike pattern for later use.

**Table 2. Spike Pattern Database**



**Exercise 3B: Stimulation of the metathoracic ganglion**

Now that you have the spiking patterns of motor input from the metathoracic ganglion to the legs, you will test whether the patterns you recorded will lead to the specific movements you observed.

1. If using the alligator clip microstimulation electrode, attach one electrode to the recording electrode already placed in the metathoracic ganglion. If using electrode pin attached to an audio cable, replace the recording electrode with the microstimulation electrode. Try to insert the electrode into the same spot.

2. Similar to Exercise 1, begin measuring the effectiveness of your microstimulation electrodes. In Table 3, record the effect of microstimulation on your cockroach prep in terms of movements. Record a brief description of movement in response to a 5 second stimulation.

**Table 3.Effect of Frequency and Amplitude on Movement**

|  |  |  |  |
| --- | --- | --- | --- |
| **Frequency** | **Min Output** | **½ Output** | **Max Output** |
| **20 Hz** |  |  |  |
| **80 Hz** |  |  |  |
| **200 Hz** |  |  |  |
| **1000 Hz** |  |  |  |
| **2000 Hz** |  |  |  |

3. Now, using the Spike Patterns generated in Exercise 3A, stimulate the metathoracic ganglia at different volumes. Record the results in Table 4. Describe the movement observed (if any), and whether this movement is the same as the corresponding movement recorded in Table 2.

**Table 4. Effect of Spike Patterns and Amplitude on Movement**

|  |  |  |  |
| --- | --- | --- | --- |
| **Spike Pattern** | **Min Output** | **½ Output** | **Max Output** |
| **Pattern 1** |  |  |  |
| **Pattern 2** |  |  |  |
| **Pattern 3** |  |  |  |
| **Pattern 4** |  |  |  |
| **Pattern 5** |  |  |  |

**DISCUSSION QUESTIONS**

1. In this experiment you tested the responsiveness of the cockroach legs to a wide range of frequencies and amplitudes. Was there any commonality in the types of frequencies and/or amplitudes that caused the most responsiveness? Why do you think that might be?

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1. Were you able to identify any spiking patterns that led to motor movements? If so, what were the characteristics of these patterns? What types of movements were you able to find these patterns for? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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1. When you stimulated the metathoracic ganglia with spike patterns that you had observed during leg movements, did it generate any leg movements? Were these leg movements the same as the ones you had observed while recording those spike patterns? Why do you think this is? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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